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The Effect of Prehatch and Posthatch Exposure to Cadmium on Salinity Tolerance of Larval Grass Shrimp, 'Palaemonetes pugio'

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16. ABSTRACT
Groups of embryonic grass shrimp, Palaemonetes pugio, were exposed to 0.1 and 0.3 mg/l cadmium at 30 ppt salinity and 25°C for the last 1, 4, or 8 days prior to hatching. Other groups of embryos were cultured in uncontaminated seawater.

Prehatch exposure to cadmium was found to have no additive effect on the sensitivity of the larvae to cadmium exposure and salinity stress for 14 days after hatching. Only one group of larvae, exposed to 0.1 mg/l cadmium for 4 days before hatching and transferred to 10 ppt salinity water containing 0.1 mg/l cadmium after hatching, showed a significant (X^2, $P < 0.05$) decrease in survival, compared to control survival. No significant decreases in survival were observed for any larvae transferred to 15 and 30 ppt salinity at a pre- and posthatch cadmium concentration of 0.1 mg/l.

At a pre- and posthatch cadmium concentration of 0.3 mg/l, significant decreases in survival were observed for all of the larvae transferred to 10 and 15 ppt salinity after hatching. Significant decreases in survival were observed for only 2 of the groups exposed before hatching and transferred to 30 ppt salinity and 0.3 mg/l cadmium after hatching.

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The Effect of Prehatch and Posthatch Exposure to Cadmium on Salinity Tolerance of Larval Grass Shrimp, *Palaemonetes pugio*

ABSTRACT: Groups of embryonic grass shrimp, *Palaemonetes pugio*, were exposed to 0.1 and 0.3 mg/l cadmium at 30 ppt salinity and 25°C for the last 1, 4 or 8 days prior to hatching. Other groups of embryos were cultured in uncontaminated seawater.

Prehatch exposure to cadmium was found to have no additive effect on the sensitivity of the larvae to cadmium exposure and salinity stress for 14 days after hatching. Only one group of larvae, exposed to 0.1 mg/l cadmium for 4 days before hatching, and transferred to 10 ppt salinity water containing 0.1 mg/l cadmium after hatching, showed a significant (X^2, $P < 0.05$) decrease in survival, compared to control survival. No significant decreases in survival were observed for any larvae transferred to 15 and 30 ppt salinity at a pre- and posthatch cadmium concentration of 0.1 mg/l.

At a pre- and posthatch cadmium concentration of 0.3 mg/l, significant decreases in survival were observed for all of the larvae transferred to 10 and 15 ppt salinity after hatching. Significant decreases in survival were observed for only 2 of the groups exposed before hatching and transferred to 30 ppt salinity and 0.3 mg/l cadmium after hatching.

Introduction

The grass shrimp, *Palaemonetes pugio*, has a geographic range that extends from Massachusetts to Texas (Williams 1965). Egg bearing females are abundant from April through mid-October in estuaries throughout the range.

Palaemonetes are susceptible to both adverse environmental conditions and heavy metal toxicity. Broad (1957), and Broad and Hubschman (1962) determined that survival of *P. pugio* was best at approximately 25°C and salinities above 20 parts per thousand (ppt), but poor at salinities of 10 ppt or less.

In a survey of cadmium toxicity to marine organisms, Eisler (1971) ranked adult *P. vulgaris* as the third most sensitive organism tested, with a 96 hour LC50 of 0.42 mg/l cadmium. Larval *P. vulgaris* have also been shown to be sensitive to other heavy metals. Delayed effects of 48 hours sublethal exposure to mercury included, reduced survival to the postlarval stage, delayed molting, increased numbers of instars and morphological deformities (Shealy and Sandifer 1975).

The synergistic effect of temperature and salinity stress in combination with the toxic effect of mercury has been demonstrated for larvae of another marine crustacean, the fiddler crab, *Uca pugilator*, (DeCoursey and Vernberg 1972; Vernberg et al. 1973).

Our study was designed to assess the effect of exposure of developing *P. pugio* embryos to cadmium on subsequent larval sensitivity to combined cadmium and salinity stress.

Materials and Methods

Gravid females were collected during July and August, 1974 from We Creek, a tidal tributary of the North Edisto River estuary in South Carolina. Collections were made with a 10 m × 5 mm mesh bag seine. Water temperatures ranged from 21 to 29°C, the salinity from 18 to 29 ppt. Females were maintained in an aerated 40-liter glass aquarium (water temperature 25°C, salinity 30 ppt) for 2 to 6 days prior to use in tests. They were fed *Artemia* nauplii twice daily.

Cadmium Exposure and Salinity Stress

In one series of tests, developing embryos were exposed to 0.1 or 0.3 mg/l cadmium at 30 ppt salinity for the last 1, 4, or 8 days prior to hatching. Newly hatched larvae were transferred to 10, 15 or 30 ppt salinity seawater which also contained 0.1 or 0.3 mg/l cadmium. A second series of tests determined the effect of cadmium toxicity and salinity stress on the survival of larvae with no prehatch cadmium exposure. Survival of control embryos which developed at 30 ppt salinity and were transferred to uncontaminated 10, 15 or 30 ppt salinity seawater after hatching was also determined for comparison with the survival of cadmium exposed larvae.

Prehatch Cadmium Exposures

For prehatch cadmium exposures, gravid females were held individually in acrylic plastic tubes (90 mm long × 40 mm diameter) in a recirculating bioassay system (Fig. 1). Water flow through the plastic holding tubes ranged from 7 to 9 liters per hour. Aliquots of a 1.0 g/l stock solution of reagent grade (ACS) cadmium, as $CdCl_2 \cdot 2\frac{1}{2}H_2O$, prepared in distilled water, were added to two of the systems to give nominal concentrations of 0.1 and 0.3 mg/l cadmium. A third system was used for controls and for obtaining larvae which were used for posthatch cadmium exposure only.

All three bioassay systems were immersed in a water bath which maintained the temperature at 25 ± 1°C. The photoperiod was 14 hours light and 10 hours dark. Fluorescent "cool white" lamps provided 1800–2000 lux of illumination.

As the larvae hatched, they were carried by the water flow from the adult holding tubes into acrylic plastic rearing cups (Fig. 1). Each cup (60 mm deep × 75 mm diameter) had a plankton netting bottom (125 μ) which retained the larvae. To prevent excessive water turbulence in the rearing cups, water flow through the systems was reduced to 2 to 3 liters per hour after a hatch occurred.

Preliminary tests indicated that exposure of developing embryos to 0.1 or 0.3 mg/l cadmium for up to 11 days had no effect on the survival of the newly hatched larvae at 30 ppt salinity. However, occasional hatches were observed for controls and for embryos exposed to cadmium in which the larvae showed poor survival and reduced activity. Because of this, hatches in which mortality exceeded 10 percent were not used. Similar observations on the variability of crustacean larval fitness and selection of hatches on the basis of survival and behavior have been made by DeCoursey and Vernberg (1972).

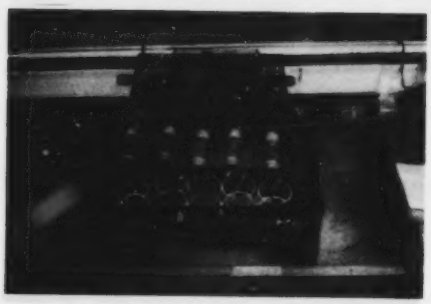

Fig. 1. Recirculating bioassay system for prehatching exposure of developing grass shrimp embryos to cadmium. (A) Gravid females were placed in each of the 5 exposure tubes. (B) As the larvae hatched they were carried by the water flow into the rearing cups.

Posthatch Cadmium Exposure

After hatching, three groups of 15 larvae (less than 12 hours old) were pipetted into individual 60 mm diameter glass culture dishes (1 larva per dish) that contained 15 ml of 10, 15, or 30 ppt salinity seawater. For bioassays with larvae exposed to cadmium before hatching and those exposed only after hatching (no prehatch exposure), the water was treated with 0.1 or 0.3 mg/l cadmium. No cadmium was added to water in control dishes.

Culture dishes were maintained in constant temperature boxes at 25°C with a 14 hour light and 10 hour dark photoperiod (1400 lux). Checks for death were made every 24 hours. Solutions in the culture dishes were changed every 3 or 4 days. Larvae were fed *Artemia* nauplii daily.

Tests were terminated after 14 days. Chi-squared analyses (2×2 Contingency table, $P < 0.05$) were performed to determine if significant differences existed between survival of cadmium exposed and control larvae.

Cadmium concentrations in the recirculating bioassay systems and culture dishes were measured randomly with a Perkin-Elmer Model 403 Atomic Absorption Spectrophotometer[1] (Table 1).

Results

Prehatching Exposures

Prehatch exposure of grass shrimp to 0.1 mg/l cadmium for 1, 4, and 8 days at 30 ppt salinity and 25°C had no additive effect on their survival when subjected to 0.1 mg/l cadmium at 10, 15 or 30 ppt salinity for 14 days after hatching.

At 10 ppt salinity, decreased survival of larvae with 4 days of prehatch exposure to 0.1 mg/l cadmium and those with no prehatch exposure (0 days) resulted from the combined effect of salinity stress and posthatch cadmium toxicity (Fig. 2). Chi-squared analysis ($P < 0.05$) indicated that decreases in the number of survivors was significant only for the group exposed to cadmium for 4 days prior to hatching.

Some decreases in survival were also observed for bioassays in which larvae were tranferred to 15 ppt salinity and 0.1 mg/l cadmium after hatching. None of these decreases were statistically significant. Larvae maintained in an optimal rearing salinity of 30 ppt and 0.1 mg/l cadmium during the pre- and posthatch intervals showed excellent survival.

Larvae exposed to 0.3 mg/l cadmium at 30 ppt salinity and 25°C also showed no obvious decreases in survival which could be attributed to the prehatch exposure interval. For bioassays in which larvae were transferred to 10 ppt salinity at 0.3 mg/l cadmium after hatching, statistically significant decreases in the number of survivors occurred in all groups (1, 4 and 8 days of prehatch exposure, and those with no prehatch exposure, 0 days) (Fig. 2).

Significant decreases in survival were also observed for all of the groups of larvae exposed to 0.3 mg/l cadmium at 30 ppt salinity before hatching and then transferred to 15 ppt salinity after hatching. However, only those larvae exposed to 0.3 mg/l cadmium at 30 ppt salinity for 1 and 4 days before hatching and transferred to 0.3 mg/l cadmium at 30 ppt salinity after hatching showed significant decreases in survival.

Discussion

Several recent studies have shown that suboptimal temperature and salinity regimes may affect the survival of marine crustaceans (Costlow et al. 1960; Costlow and Monroe 1962; Sandifer 1973).

DeCoursey and Vernberg (1972) and Vernberg et al. (1973) have demonstrated changes in the physiological responses of larval *Uca pugilator* exposed to 1.8 ug/l mercury under suboptimal temperature and salinity regimes. Indicators of stress included reduced survival, alteration of oxygen consumption and modified phototactic response.

In research with larval *Palaemonetes vulgaris*, Shealy and Sandifer (1975) learned that mercuric chloride was acutely toxic at concentrations of 0.056 mg/l mercury or greater. Several delayed effects of 48 hour exposure of newly hatched *P. vulgaris* larvae to sublethal mercury concentrations <0.0056 mg/l were observed. These included, reduced survival to the postlarval stage, delayed molting, extended developmental time and morphological deformities.

TABLE 1. Nominal concentrations of cadmium added and measured ranges in recirculating bioassay systems and salinity tolerance tests.

Expt. Regime	Sal. ppt	Nominal conc. mg/l	Measured conc. mg/l
Recirculating bioassay systems	30	0.10	0.10–0.12
	30	0.30	0.29–0.33
Salinity tolerance tests	10	0.10	0.09–0.11
	15	0.10	0.10–0.11
	30	0.10	0.10–0.11
	10	0.30	0.28–0.30
	15	0.30	0.29–0.30
	30	0.30	0.27–0.31

[1] Mention of trade names does not imply endorsement by the U.S. Environmental Protection Agency.

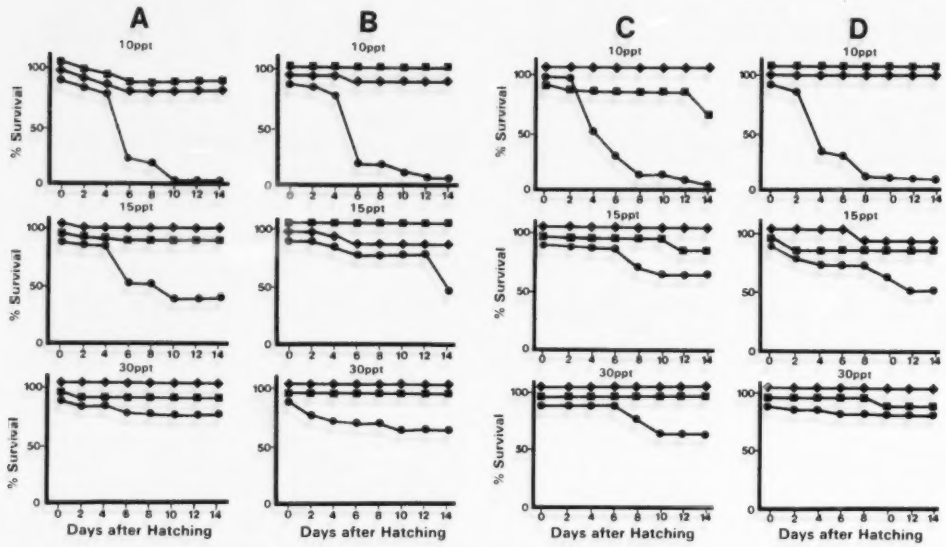

Fig. 2. Survival of larval grass shrimp exposed to cadmium. A – no prehatch exposure (0 days), B – 1 day, C – 4 days, D – 8 days of prehatch exposure. Key: Control – ◆, 0.1 mg/l Cd – ■, 0.3 mg/l Cd – ●.

In the present study we began exposure of embryonic *P. pugio* to cadmium up to 8 days prior to hatching. This prehatch exposure of developing embryos to cadmium had no effect on their response to suboptimal salinity regimes in combination with cadmium toxicity after hatching.

Two membranes which isolate the developing palaemonid embryo from the surrounding environment (Davis 1965) apparently limited the effect of cadmium exposure during development.

Decreases in survival for larvae exposed to 0.1 and 0.3 mg/l cadmium at low salinity regimes (10 and 15 ppt) indicate the combined effect of salinity stress and posthatch cadmium toxicity, rather than any additive effect of exposure of developing embryos to cadmium. These results demonstrate that a pollutant stress which would not be lethal at optimal salinities may become lethal if encountered at suboptimal salinity regimes.

Literature Cited

Broad, A. C. 1957. Larval development of *Palaemonetes pugio* Holthuis. *Biol. Bull.* 112: 144–161.

———, and J. H. Hubschman. 1962. A comparison of larvae and larval development of species of Eastern U. S. *Palaemonetes* with special reference to the development of *Palaemonetes intermedius* Holthuis. *Amer. Zool.* 2: 394–395.

Costlow, J. D., Jr, C. G. Bookhout, and R. J. Monroe. 1960. The effect of salinity and temperature on *Sesarma cinereum* Bosc reared in the laboratory. *Biol. Bull.* 118: 183–202.

———, and R. J. Monroe. 1962. Salinity-temperature effects on the larval development of the crab, *Panopeus herbstii*, reared in the laboratory. *Physiol. Zool.* 35: 79–93.

Davis, C. C. 1965. A study of the hatching process in aquatic invertebrates. XIV. An examination of hatching in *Palaemonetes vulgaris* Say. *Crustaceana* 8(3): 233–238.

DeCoursey, P. J., and W. B. Vernberg. 1972. Effect of mercury on survival, metabolism and behavior of larval *Uca pugilator* Brachyura. *Oikos* 23: 241–247.

Eisler, R. 1971. Cadmium poisoning in *Fundulus heteroclitus* (Pisces: Cyprinodontidae) and other marine organisms. *J. Fish. Res. Bd. Canada* 28: 1225–1234.

Sandifer, P. A. 1973. Effects of temperature and salinity on larval development of grass shrimp, *Palaemonetes vulgaris*. *Fish. Bull. U. S. Fish Wildl. Serv.* 71: 115–121.

Shealy, M. H., and P. A. Sandifer. 1975. Effects of mercury on survival and development of the larval grass shrimp *Palaemonetes vulgaris*. *Mar. Biol.* 33: 7–16.

Vernberg, W. B., P. J. DeCoursey, and W. J. Padgett. 1973. Synergistic effects of environmental variables on larvae of *Uca pugilator*. *Mar. Biol.* 22: 307–312.

Williams, A. B. 1965. Marine decapod crustaceans of the Carolinas. *Fish. Bull. U. S. Fish Wild. Serv.* 65: 1–298.

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